virus and not of SV-40; it was concluded that the SV-40 genome was included within the protein coats of adenovirus. We now report on further passages of the contaminant SV-40 in the presence of adenovirus 4 and antiserum to SV-40.

Details concerning antiserum, virus, and methods of viral assay have been described (*1*). The antiserum had a titer of 1:20,000 against 100 $TCID_{50}$ (tissue-culture infective doses, 50 percent effective) of SV-40, and was present in a concentration of 1:250 in passages No. 7 through No. 11. No additional antiserum was added to passage No. 12, although in this passage antiserum was carried over from passage No. 11. In addition to titration in cell cultures of *Cercopithecus aethiops* kidney cultures, the various passages were titrated in human-embryo kidney cells to assess the true concentration of the adenovirus (*2*). The 6th and the 12th SV-40 passages were in cercopithecus kidney cells, while the intervening passages were in human-embryo kidney cells. Frozen, thawed, and centrifuged virus suspension was added to SV-40 antiserum (diluted 1 : 5), and the mixture was incubated at 36°C for 0.5 hour prior to inoculation into the next succeeding culture. As a control, $10^{5.5}$ $TCID_{50}$ of SV-40, obtained by end-dilution of passage No. 6 in cercopithecus cells and free of any large amounts of adenovirus 4, were treated in a manner analogous to that in passages No. 7 through No. 12; that is, all passages were made in the presence of SV-40 antiserum.

Table 1 shows that the titers of SV-40 and adenovirus 4 remained relatively constant in the seven passages shown. The cumulative dilution of the sample from passage No. 6, by the time it reached passage No. 12, was over a billion fold. Thus, not only did SV-40 resist the action of antiserum to SV-40 after it had replicated in the presence of adenovirus 4, but it also multiplied at a seemingly constant rate. In contrast, the SV-40 from passage No. 6, freed of adenovirus 4, had a titer of only $10^{0.3}$ $TCID_{50}$ per 5 ml in passage No. 7; none could be detected in subsequent passages. This showed that the SV-40 alone was rapidly neutralized by its homologous antiserum.

The titers of SV-40 and adenovirus 4 in the various passages bear a fixed ratio to one another, an indication that the velocity constants for the replication of both viruses are similar. Incorporation within coat proteins of adenovirus 4 may not only confer on SV-40 the phenotypic properties described (*1*), but may also enable small amounts of SV-40 to replicate in human embryo-kidney cell cultures with a facility approximating that of the adenovirus itself.

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References and Notes

1. J. M. Easton and C. W. Hiatt, *Proc. Nat. Acad. Sci. U.S.* **54**, 1100 (1965).
2. G. T. O'Conor, A. S. Rabson, I. K. Berezesky, F. J. Paul, *J. Nat. Cancer Inst.* **31**, 903 (1963); A. S. Rabson, G. T. O'Conor, I. K. Berezesky, F. J. Paul, *Proc. Soc. Exp. Biol. Med.* **116**, 187 (1964).
3. We thank Dr. J. L. Bittle, Lederle Laboratories, for provision of virus stocks and antiserum and C. A. Robinson for technical assistance.
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Major Components in the Exocrine Secretion of a Male Butterfly (Lycorea)

Abstract. *Extracts of the extrusible secretion-disseminating organs ("hairpencils") of the male of the danaid butterfly,* Lycorea ceres ceres, *from Trinidad, contain a pyrrolizidine and two aliphatic esters. An odorous component, present in trace amounts, remains unidentified. Judging from the function of "hairpencils" in a related species, the secretion may play a mediating role in courtship.*

Courtship, mating, and other social interactions of animals are frequently mediated by substances called pheromones, secreted by the animals themselves (*1*). Among the very few pheromones that have been isolated and identified are the sex attractants of the silkworm moth (I) and gypsy moth (II). No studies have been made on butterflies, despite the fact that these insects are often endowed, in one sex or the other, with exocrine glands that might logically be presumed to secrete pheromones (*2*). The males of the subfamily Danainae (family Nymphalidae) possess a pair of extrusible brushlike structures, the "hairpencils," which because of their noticeable odor have long been suspected to act as scent-disseminating organs, and which are, in fact, associated with secretory cells at their base. In the queen butterfly, *Danaus gilippus berenice*, whose courtship has recently been investigated in detail (*3*), the hairpencils are protruded by the males during aerial pursuit of the female and are brushed against her head and antennae. In response to this behavior, the female alights on available herbage, while the male hovers above her and continues to "hairpencil" her

$$CH_3(CH_2)_2CH{=}CHCH{=}CH(CH_2)_8CH_2OH$$

(I)

$$CH_3(CH_2)_5\underset{\displaystyle OCCH_3\\ \displaystyle \|\\ \displaystyle O}{CH}CH_2CH{=}CH(CH_2)_5CH_2OH$$

(II)

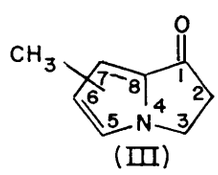
anterior end. Eventually he settles beside her and copulation occurs.

As a first step in a comparative study of the hairpencil secretion of a variety of danaid butterflies, we are reporting on the chemistry of the secretion of one of them, *Lycorea ceres ceres*, from Trinidad. The hairpencils of this species are particularly large (Fig. 1) and richly endowed with secretion.

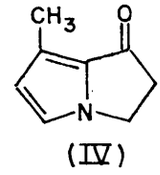
The tufts of hairs from hairpencils of about 300 live *Lycorea* males (*4*) were pulled off with forceps and extracted with methylene chloride or carbon disulfide. The infrared spectrum of this extract showed strong absorption at 3.45, 3.52, 5.77, 5.92, and 8.10 μ, and gas-liquid chromatography (GLC), on a column of 5 percent SE-30 silicone gum at 180°C, showed three major components (retention times 1.7, 10.5, and 20 minutes). Fractional sublimation gave a crystalline compound with the characteristic sweetish odor of the natural secretion. This component, mp 74° to 75°C, had infrared bands at 3.4, 5.95, and 6.45 μ. In the ultraviolet, a maximum at 288 mμ (log ϵ 4.22, based on the molecular weight of 135 determined by mass spectrometry) implied the presence of a conjugated system. These data speak for the molecular formula C_8H_9ON. A nuclear magnetic resonance spectrum at 100 Mcy/sec clearly confirmed the presence of nine protons, disposed as follows: a three-proton singlet at τ 7.80, a pair of two-proton triplets (the coupling constant $J \cong 6.5$ cy/sec at τ 7.16 and 5.87, and a pair of less well-resolved one-proton doublets ($J \cong 2.5$ cy/sec) at τ 3.91 and 3.31.

From these data, a methyl-2,3-dihydro-1*H*-pyrrolizidin-1-one structure (III) can be derived. On the basis of the coupling constant between the pyrrole protons, in good agreement with that generally observed for adjacent α and β protons (*5*), the methyl group may be assigned to carbon No. 7, and the structure of this component defined as IV; this assignment is supported by comparison with model compounds and by independent synthesis (*6*).

The physical properties of synthetic compound IV, including ultraviolet, infrared, and mass spectra, were indistinguishable from those of the natural product. However, synthetic IV lacked the characteristic odor associated with the material from the butterfly. This difference, undetectable even by mass spectroscopy, is evidently due to trace impurity, and will be exceedingly difficult to characterize.



(III)



(IV)

The two remaining major components were separated by preparative GLC. The constituent of shorter retention time showed infrared absorption at 5.77 and 8.10 μ, suggestive of an acetate function. A molecular weight of 284 by mass spectrometry indicated the composition $C_{16}H_{33}OCOCH_3$. This ester was identified as cetyl (*n*-hexadecyl) acetate (V) on the basis of comparisons by GLC, infrared, and mass spectrometry.

$CH_3(CH_2)_{15}OCOCH_3$
(V)

$CH_3(CH_2)_{17}OCOCH_3$
(VI)

The final major component, which also had infrared bands at 5.77 and 8.10 μ, showed a molecular weight (310) corresponding to the acetate of a C_{18}-unsaturated or cyclic alcohol, $C_{18}H_{33}OCOCH_3$. Catalytic hydrogenation of this ester gave a new ester, identified as stearyl (*n*-octadecyl) acetate (VI) on the basis of GLC comparison with an independently prepared sample. Permanganate-periodate oxidation of the unsaturated ester, followed by diazomethane treatment, gave methyl heptanoate (VII), identified by GLC comparison with an authentic sample, along with an acetoxy ester identified as methyl 11-acetoxyundecanoate (VIII). The formation of these fragments fixes the position of the double bond; taken along with the absence of *trans*-olefinic infrared absorption, it leads to the identification of this component as *cis*-vaccenyl acetate (IX). This structure was confirmed by inde-

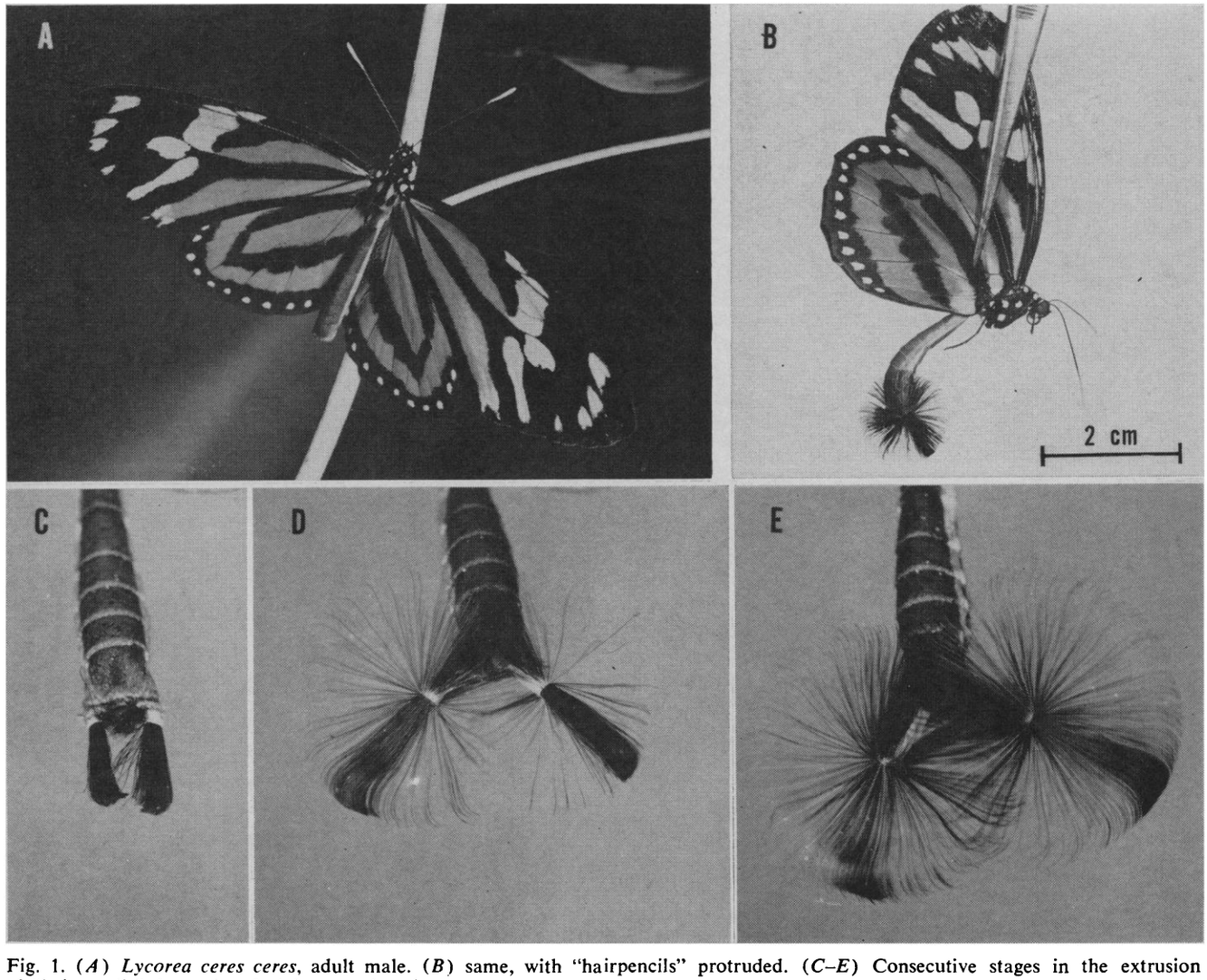


Fig. 1. (*A*) *Lycorea ceres ceres*, adult male. (*B*) same, with "hairpencils" protruded. (*C–E*) Consecutive stages in the extrusion of "hairpencils" from tip of abdomen of male.

pendent synthesis. A mixture of the three synthetic components, IV, V,

$$CH_3(CH_2)_5CO_2CH_3$$
(VII)

$$CH_3O_2C(CH_2)_{10}OCOCH_3$$
(VIII)

and IX, gave an infrared spectrum indistinguishable from that of the unfractionated hairpencil secretion.

$$CH_3(CH_2)_5-\underset{H}{\overset{|}{C}}=\underset{H}{\overset{|}{C}}-(CH_2)_{10}OCOCH_3$$
(IX)

Pyrrolizidinones have not previously been isolated from insects or other animals, although this ring system, in a lower oxidation state, is a characteristic moiety of the senecio alkaloids (*7*). In contrast, the two acetate esters bear striking, and possibly meaningful, similarity to the two known pheromones from moths (I, II).

Whether the three components (together with the elusive odorous factor) serve in "unison" to convey a single message, or whether they constitute a medley of distinct signals, some perhaps not even related to courtship (*8*), remains to be determined. Work on the behavior of *Lycorea* is hampered by the fact that this species apparently courts in dense tropical forest. Studies on the biological "meaning" of danaid hairpencil secretions might therefore best be carried out on a species such as the queen (*Danaus gilippus berenice*), which courts in a more open habitat.

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References and Notes

1. W. H. Bossert and E. O. Wilson, *J. Theoret. Biol.* **5**, 443 (1963); E. O. Wilson, *Science* **149**, 1064 (1965); ——— and W. H. Bossert, *Recent Progr. Hormone Res.* **19**, 673 (1963).
2. R. Barth, *Orgãos odoríferos dos Lepidópteros* (Parque Nacional do Itatiaia, Bull. no. 7, Rio de Janeiro, 1960); M. Jacobson, *Insect Sex Attractants* (Interscience, New York, 1965).
3. L. P. Brower, J. van Zandt Brower, F. P. Cranston, *Zoologica* **50**, 1 (1965).
4. Some of the extractions were done in Trinidad; others were done at Ithaca, N.Y., from butterflies shipped live from Trinidad in individual envelopes (the insects are extremely hardy, and survive the trip even in winter).
5. R. F. M. White, in *Physical Methods in Heterocyclic Chemistry*, A. Katritzky, Ed. (Academic Press, New York, 1963), vol 2, p. 103.
6. J. Meinwald and Y. C. Meinwald, in preparation.
7. N. J. Leonard, *Alkaloids* **1**, 108 (1950); *ibid.* **6**, 37 (1960).
8. *Lycorea*, in common with some but not all other danaid butterflies, everts its hairpencils in response to handling (Fig. 1*B*). This suggests that the secretion, or perhaps only one of its components (possibly the pyrrolizidinone), might have a defensive role.
9. Supported by grant AI-02908 from NIH (T.E. and J.M.), NIH training grant 5TI-GM-A34-02 (Chemistry, Cornell), unrestricted funds from the Upjohn Co. (T.E.), and grants 20152 and GB 2291 from NSF (L.P.B.). Jocelyn Crane, Department of Tropical Research, New York Zoological Society, H. Croze and T. Pliske, undertook the laborious task of collecting and shipping most of the butterflies. We thank Drs. A. F. Thomas and B. Willhalm, Firmenich & Cie., Geneva, for the mass spectra, and Dr. R. Pitcher, Varian Associates, for the nuclear magnetic resonance spectrum.
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Nuclear Number in the Rotifer Asplanchna: Intraclonal Variation and Environmental Control

Abstract. *The gastric glands and vitellarium of* Asplanchna *are exceptions to the rule of constancy of the number of nuclei in rotifers. The two glands of a female may have different numbers of nuclei. Nuclear number in both organs varies widely among different individuals in a single culture. The mean nuclear number characteristic of a clone may be modified by dietary changes.*

Rotifers, the rest of the Aschelminthes, and certain other invertebrate phyla show the phenomenon of eutely (*1*): each adult organ contains a constant, species-specific number of cells (or nuclei, in syncytial tissues). The gastric glands and vitellarium of rotifers of the genus *Asplanchna* are exceptions to this principle. We have studied the variability in number of nuclei in gastric glands of individual animals, and the number in the glands and vitellarium among individuals of a clone in identical environments. We have also demonstrated an environmental influence on nuclear number in both organs.

All studies were done on females, as the vitellarium is lacking and the gastric glands are degenerate in the male. Most or all of the females examined were amictic. The rotifers were grown in a baked-lettuce infusion (*p*H 7.4–7.6) and fed *Paramecium aurelia* (Medium 29) (*2*). In some experiments, cultures grown on Medium 29 were compared with cultures fed green *Paramecium bursaria* or *Eudorina elegans* suspended in the baked-lettuce infusion. In one experiment, animals were fed *Paramecium bursaria* in Gilbert's solution (*3*). All cultures were kept at 23°C in constant light, either in plastic tissue culture dishes or in glass culture tubes.

We have examined three species of *Asplanchna*. *A. Girodi* from Tennessee (one clone) and *A. brightwelli* (*sensu latissimo*) from Indiana and Tennessee (four inbred clones) have been described (*2*). Data from the Indiana and Tennessee stocks were identical and are considered together. The most extensive experiments were done with clone 7B1 from Pennsylvania and with its offspring by selfing (clone 7B1-1). This clone has been identified as *A. sieboldi* (*4*); it is morphologically, physiologically, and serologically similar to, but not identical with, the Indiana and Tennessee *A. brightwelli*.

For nuclear counts, a few live females were placed in a small drop of culture fluid on a slide and covered with a coverslip, so that the animals were slightly squashed. The nuclei were counted under a phase objective at a magnification of 400 or 500×. We counted nuclei only in those organs in which it was reasonably certain that all nuclei were visible. Any errors arising from failure to count all nuclei or (in gastric glands) from counting nuclei in other, overlying tissues were insignificant compared to the variability observed.

A large proportion (70 to 80 percent) of the females of all stocks had different numbers of nuclei in their two

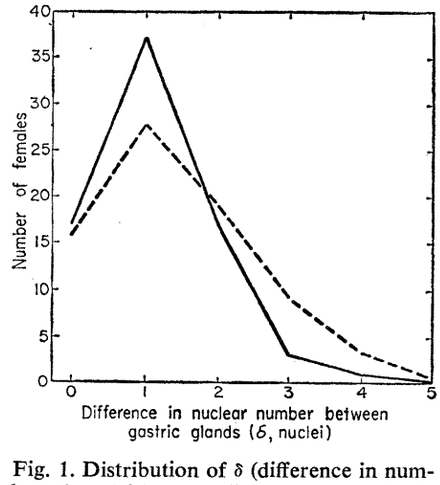

Fig. 1. Distribution of δ (difference in number of nuclei between the two gastric glands of one female) in pooled data from two samples of clone 7B1-1 fed *Paramecium aurelia*. Solid line: observed distribution. Broken line: distribution expected if the population of glands were paired randomly in females.